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TRITION UPON THE COMPOSITION OF THE TIS-
SUES AND PROTEINS OF THE WHITE RAT

BY WILLIAM C. LEE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRE-
MENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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THE EFFECT OF FASTING, REFEEDING, AND OF VARIATIONS IN THE CYSTINE CONTENT OF THE DIET ON THE COMPOSITION OF THE TISSUE PROTEINS OF THE WHITE RAT

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While certain investigators have maintained that the composition of the proteins of the tissues is influenced by the state of nutrition of the organism, others have concluded that marked variations from the normal dietary do not alter significantly the chemical constitution of these proteins. The greater part of the evidence is indirect, based upon studies of the urinary N:S ratios, upon balance studies of these two elements, and upon analyses of the entire body or of certain individual tissues under varying conditions of nutrition. Separation and analyses of the tissue proteins themselves have seldom been attempted. The earlier literature is well summarized by Cathcart (1).

Di Frisco (2), who found that the N:S ratio of the entire body of the white rat was not changed after fasting or after maintenance on a diet low in sulfur (starch or starch and gelatin), concluded that the tissue proteins are constant in composition, unaffected by the diet. No relationship between the proteins of the egg and the diet of the hen was evident (3, 4), although certain earlier work had indicated variations in the proteins of the egg white of pigeons, associated with dietary differences (5, 6). In a series of recent papers (7), Schenck and Wollschitt have studied the influence of age, diet, and the ingestion of hormones and toxic substances on the composition of tissue proteins of the white rat and have concluded that these factors influence the constitution of the proteins of the tissues. Cystine and tryptophane in particular are considered to be subject to variation. After fasting, the tryptophane contents of the proteins of liver and of muscle were decreased,

while a slight but definite increase in the cystine content of the muscle protein was observed.

In view of the indispensability of certain amino acids, it appeared probable that, if the fasting organism did draw upon the proteins of certain tissues for one or more amino acids, the essential amino acids would be the first units to be removed from the molecule. If partial cleavage of the molecule to yield these acids could be effected, the changes should be apparent upon analysis of the tissue protein in question. Thus, if cystine were split from the protein molecule, the percentages of both cystine and sulfur should be lowered, while the N:S ratio should increase. Moreover, since cystine is required by the young white rat for growth, it seemed possible that lack of an adequate supply of cystine over a considerable period of time might result in some alteration in the composition of the tissue proteins. It should also be possible to determine whether the growing rat receiving a considerable excess of cystine in the diet stores in its tissues an appreciable amount of the ingested amino acid, either as the free acid itself, or as protein with a cystine content higher than normal. The purpose of the present study is to present analyses of the tissue proteins (liver, muscle, kidney) from white rats, which had been fasted, refed, or fed for prolonged periods on diets high or low in their content of cystine. No evidence of any significant alteration of the constitution of the tissue proteins was obtained.

EXPERIMENTAL

Five series of experiments were carried out with adult male rats, 175 to 275 gm. in weight. In each series the animals were placed in individual cages and supplied with a diet of purified food substances, complete in all factors, for at least a week. They were then divided into three groups, the first of which continued to receive the diet. The second group was fasted for 48 or 72 hours, while the third group, after a fasting period of 48 hours, was allowed to consume the control diet at will for 24 hours. The animals were killed and the tissues analyzed at the end of the experimental periods indicated and the groups are designated as the normal, fasted, and refed groups, respectively. A somewhat similar experiment was carried out with a litter of ten young rats, but the influence of refeeding subsequent to fasting was not determined.

The basal diet used in our experiments was the cystine-deficient diet described by du Vigneaud and his coworkers (8) as follows: casein, 8 per cent; white dextrin, 31 per cent; Osborne-Mendel salt mixture, 4 per cent; agar, 2 per cent; lard, 19 per cent; cod liver oil, 5 per cent; and milk vitamin concentrate,¹ 16 per cent. In the experiments described above, this was supplemented by the addition of cystine in amounts adequate to insure normal maintenance and growth (0.4 to 1.0 per cent).

Two paired feeding experiments were made with young rats. In one of these litter mates were used; in the other, the animals were selected from three litters of approximately the same age. The usual procedure was followed, the animals being paired so far as possible as to sex and weight. One animal of each pair was allowed to consume the basal diet described above without any cystine supplement, at will, while its control received the same amount of the basal diet supplemented by adequate cystine (0.4 or 0.75 per cent). The experiments continued for 3 and 2 months respectively and were terminated by killing the animals and analyses of the tissues. In all experiments, daily records of food intake and weight were made.

For the analyses of the tissues the animals were killed with chloroform and the tissues removed as rapidly as possible. The liver and kidneys were cut into small pieces with scissors before analysis, while the muscle tissue was ground in a small meat chopper prior to sampling. The amount of kidney and muscle (hind and fore limbs) was so small that it was frequently necessary to combine the tissues of two animals for the analyses, particularly in the paired feeding experiments. It was usually possible to analyze each liver separately.

Moisture was determined by the method recommended by Puckett and Wiley (9), the dried samples being subsequently ashed in a muffle furnace. Total nitrogen was determined by the usual modified Kjeldahl method and total sulfur by wet ashing with concentrated nitric acid followed by the usual oxidation with the Denis reagent.

The protein content of the tissue was determined by the method

¹ The vitamin concentrate was generously supplied by Dr. G. C. Supplee of the Research Laboratories of the Dry Milk Company, before the product was available commercially.

of Janney (10). The combined alcohol-water extract, obtained from the coagulation and extraction of each protein sample in the Janney procedure, was subsequently evaporated to a volume of 100 cc. and aliquot portions were analyzed for total nitrogen and sulfur by the methods employed with the fresh tissues.

The proteins obtained by the Janney procedure from each tissue from the rats of identical experimental groups were combined for the protein analyses. Total nitrogen and sulfur were determined by the methods already stated. In some cases, however, the amount of protein available for analysis was so small that the micro-Kjeldahl method of Pregl was substituted in the determination of total nitrogen. Cystine, tyrosine, and tryptophane² were determined colorimetrically by the methods of Folin and Marenzi (11, 13). Labile sulfur was estimated by the method of Zahnd and Clarke (14). Moisture and ash were determined by drying the samples to constant weight at 100–105° followed by ashing in a muffle furnace. In a few cases moisture and ash were estimated by the micromethods of Pregl.

DISCUSSION

The result obtained with adult rats are presented in summarized form in Tables I and II. Similar values were obtained in experiments with well fed and fasted young rats; but since these studies were less extensive, owing to the fact that with smaller animals the amount of material available for analysis was limited, and since the results were essentially confirmatory of the experiments with adult animals, the data are not presented in detail.

The data obtained for muscle and kidney afford no evidence in support of the theory that nutritive conditions influence the composition of the tissue proteins. The percentages of protein in muscle (Table I) were essentially the same as those reported by Schenck and Wollschitt (7), although these workers interpret their results as indicating a slight decrease in the protein content of the muscle in fasting. Contrary also to the findings of the German investigators (7), no alterations in the cystine and tryptophane

² In the calculation of the tryptophane content of the proteins, the factor 0.843 was used as the divisor not as the multiplier. This error in the calculation of the paper of Folin and Marenzi (11) has recently been pointed out by von Deseö (12).

contents of the muscle proteins were observed in fasting (Table II). It will be noted that the labile sulfur fraction of the muscle proteins was slightly less than the cystine sulfur. In the analyses of various proteins reported by Zahnd and Clarke (14), the labile sulfur values were greater or approximately equal to the cystine sulfur. Whether this difference in the relationship between cystine sulfur and labile sulfur is related to the chemical structure and composition of the proteins of muscle or to some factor of tech-

TABLE I

Analysis of Fresh Tissue and Tissue Extracts of Adult Rats under Varying Conditions of Nutrition

	Tissue	Fresh tissue						Extract*		
		Protein	Total N	Total S	N:S	Moisture	Ash	Total N	Total S	N:S
		per cent	per cent	per cent		per cent	per cent	per cent	per cent	
Group I. Normal diet, 14 rats	Liver	22.0	2.8	0.23	12.2	70.2	1.29	0.27	0.05	5.4
	Muscle	19.2	3.4	0.24	14.1	73.5	1.23	0.42	0.06	7.0
	Kidney†	17.5	2.9	0.25	11.6			0.33	0.06	5.5
Group II. Fasted, 16 rats	Liver	21.6	3.5	0.26	13.4	71.0	1.40	0.28	0.05	5.6
	Muscle	19.6	3.5	0.25	14.0	72.9	1.22	0.49	0.07	7.0
	Kidney†	17.6	3.1	0.24	12.9			0.37	0.05	7.4
Group III. Fasted and refed, 14 rats	Liver	22.1	2.6	0.22	11.8	69.8	1.19	0.27	0.08	3.4
	Muscle	19.3	3.4	0.25	13.6	73.7	1.19	0.41	0.07	5.9
	Kidney†	17.3	3.0	0.25	12.0			0.35	0.07	5.0

* Total nitrogen and sulfur of the extract are calculated as percentages of the original tissue. See text for the preparation of the extract.

† The values for nitrogen and sulfur of the kidney are the averages of analyses from eight animals.

nique cannot be stated. The same relationship was observed in the experiments with young rats to be discussed later (Table IV).

The most striking change observed in the analyses of the liver is the marked increase in total nitrogen (approximately 23 per cent) in fasting animals. Smaller increases in the values for total sulfur were noted. These changes, although less marked than the changes in total nitrogen, were obtained so consistently that we believe that they are beyond the range of experimental error. A similar increase in total nitrogen of the liver in fasting has been

reported previously (15, 16). We have not observed the diminution in the water content of the fasting liver reported by Dittmar (16), our results which indicated no change being confirmatory of the observations of Puckett and Wiley (9).

Analysis of the liver proteins revealed exceptionally low values for total nitrogen and sulfur in the normal and refed groups, while

TABLE II

Composition of Proteins of Tissues of Adult Rats under Varying Nutritive Conditions

All values are calculated on an ash-free, moisture-free basis.

Nutrition	Source of protein	Total N	Total S	N:S	Cys-tine	Cys-tine S	Cys-tine S of total S	Tyro-sine	Tryp-to-phane
		per cent	per cent		per cent	per cent	per cent	per cent	per cent
Normal	Liver	13.3	0.76	17.5	1.56	0.42	55.3	3.78	1.37
	Muscle	16.1	0.98	16.4	1.15	0.31	31.6*	4.49	1.14
	Kidney	15.8	1.04	15.2	1.79	0.48	46.2	4.74	1.66
Fasting	Liver	16.0	0.96	16.6	1.88	0.50	52.1	4.63	1.73
	Muscle	16.2	0.99	16.3	1.11	0.30	30.3*	4.47	1.16
	Kidney	15.8	1.01	15.6	1.81	0.48	47.5	4.64	1.65
Fasting and refeeding	Liver	13.1	0.74	17.8	1.56	0.42	56.7	3.86	1.29
	Muscle	16.0	0.97	16.5	1.12	0.30	30.9*	4.32	1.20
	Kidney	16.0	1.04	15.4	1.93	0.52	50.0	4.88	1.64
Normal	Liver†	14.49	0.93	15.5	1.56	0.42	45.1	4.34	1.39
Fasting	" †	15.93	1.01	15.7	1.66	0.44	43.5	4.86	1.51

* Labile sulfur (as determined by the method of Zahnd and Clarke (14)) comprised 25.5, 25.3, and 26.8 per cent of the total sulfur of the muscle proteins under conditions of normal nutrition, fasting, and fasting and refeeding respectively.

† These values were obtained on the analysis of liver protein prepared by the Janney procedure and specially treated to insure extraction of non-protein material as described in the text.

the figures for the fasted group were essentially the same as those obtained for muscle protein. These low values suggested that the method of extraction used, which Janney (10) applied only to muscle tissue, was inadequate for the complete removal of glyco-gen and other substances soluble in hot alcohol and water from liver tissue. The livers of the fasting animals presumably would

have already lost a considerable part of these extractable materials in the course of fasting. Therefore, all the samples of liver protein as obtained by the Janney procedure were finely pulverized and extracted with boiling water until the extracts were completely free from opalescence, followed by the usual 18 hour extraction with alcohol. The aqueous extracts obtained from the proteins of the normal and refed rats gave strongly positive tests for glycogen, while those of the proteins of fasted animals were negative. The figures obtained in the analysis of the reextracted proteins³ are shown in Table II. The analyses of the liver protein of the fasted group were still higher than those of the normal and refed groups, in spite of the prolonged reextraction. The values for the normal and refed animals check within the limits of experimental error. The total nitrogen content of the protein of the fasted group was approximately 20 per cent higher than that of the well fed group; total sulfur, cystine, tyrosine, and tryptophane were likewise higher, to the extent of approximately 26, 20, 22, and 26 per cent respectively; *i.e.*, the percentage contents of each of these constituents of liver protein in fasting were increased to approximately the same extent. It would appear from this observation either that the Janney procedure is inadequate for the purification of liver protein, or that the protein in the tissue of the well fed animal is bound to some non-protein prosthetic group, which is split from the molecule in the course of fasting, the protein itself undergoing no change.

That the former explanation is probably correct was shown in an experiment in which six well fed and an equal number of fasted adult rats were used. The animals were anesthetized with chloroform, suspended, and decapitated in order to allow as much blood as possible to drain from the body. The livers were then removed and sliced into hot alcohol, and the tissue was thoroughly ground in a mortar. This was followed by repeated extraction with boiling water, until the filtrates were no longer opalescent, after which the usual extraction with 95 per cent and absolute alcohol was car-

³ On similar extraction of the muscle proteins, no glycogen tests were obtained in the extracts and the proteins gave essentially the same results on analysis as before extraction. Values in Table II for muscle and kidney protein are the values obtained on analysis of the protein as extracted in the original procedure of Janney.

ried out. The proteins were then dried, pulverized finely, and subjected to the same extraction process as the fresh tissue. This was repeated once more, after which the protein samples from the two groups were analyzed. The results are shown in the last two sections of Table II. Although the values for liver protein after fasting were somewhat higher than those of the protein after feeding, the differences were considerably less than those obtained in the previous experiments and suggest that still further purification might have resulted in the elimination of these differences.

TABLE III

Analysis of Fresh Tissue and Tissue Extracts of Young Growing Rats Maintained upon Diets Varying in Content of Cystine

Each group comprised nine rats.

	Tissue	Fresh tissue						Extract*		
		Protein	Total N	Total S	N:S	Moisture	Ash	Total N	Total S	N:S
		per cent	per cent	per cent		per cent	per cent	per cent	per cent	
Group I. Cystine-deficient diet	Liver	21.1	2.9	0.20	14.5	70.9	1.34	0.33	0.04	8.3
	Muscle	17.8	3.2	0.21	15.2	71.3	1.18	0.39	0.05	7.8
	Kidney	17.6	2.9	0.21	13.8			0.42	0.08	5.3
Group II.† Cystine-supplemented diet	Liver	22.3	2.9	0.22	13.1	70.4	1.35	0.30	0.08	4.1
	Muscle	18.3	3.3	0.25	13.2	71.3	1.14	0.41	0.11	3.7
	Kidney	16.8	2.9	0.25	11.6			0.42	0.15	2.8

* Total nitrogen and sulfur of the extract are calculated as percentages of the original tissue. See text for the preparation of the extract.

† Each animal of Group II was paired as to food consumption with the corresponding animal of Group I.

Although the evidence is not entirely convincing in the case of liver protein, it appears that fasting does not alter to any appreciable extent the composition of the proteins of muscle, kidney, and liver tissue of the white rat. A more detailed investigation of the proteins of the liver is planned.

The results obtained in two series in which young rats were maintained on cystine-deficient and cystine-supplemented diets by the paired feeding methods are presented in Tables III and IV. In all cases, the growth made by the animal receiving the cystine-

supplemented diet was satisfactory and distinctly better than the growth of its paired control fed a diet deficient in cystine. Weekly increases in weight of typical pairs were 2.7 and 5.2, 2.6 and 4.8, 2.5 and 5.2, 3.0 and 7.3 gm. respectively.

The most striking difference in the composition of fresh muscle was the higher sulfur content of this tissue of the rats receiving the cystine supplement with a correspondingly lower N:S ratio. The explanation for this appears to lie in the presence of sulfur in non-protein form since the sulfur content of the extracts of the muscle from the same animals was also high. This suggests a

TABLE IV

Analyses of Proteins of Tissues of Young Growing Rats Maintained upon Diets Varying in Content of Cystine

All values are calculated on an ash-free, moisture-free basis.

Nutrition	Source of protein	Total N	Total S	N:S	Cys-tine	Cys-tine S	Cys-tine S of total S	Tyro-sine	Tryp-to-phane
		per cent	per cent		per cent	per cent	per cent	per cent	per cent
Cystine-deficient diet	Liver	13.7	0.81	16.9	1.54	0.41	50.6	4.40	1.37
	Muscle	16.2	0.92	17.6	1.10	0.29	31.5*	4.59	1.31
	Kidney	16.2							
Cystine-supplemented diet	Liver	13.2	0.76	17.4	1.62	0.43	56.6	4.03	1.31
	Muscle	16.2	0.88	18.3	1.15	0.31	35.2*	4.46	1.25
	Kidney	16.5							

* Labile sulfur (14) comprised 29.3 and 30.7 per cent of the total sulfur of the muscle proteins under conditions of the absence and presence of adequate amounts of dietary cystine respectively.

temporary storage of cystine or some other form of non-protein sulfur in the tissue. Similar differences in the sulfur contents of other tissues of the two groups are apparent. The cystine content of the muscle proteins was, however, essentially the same in the two groups. In fact, no significant differences in any of the constituents of the protein molecule studied could be observed in the two groups. It appears, therefore, that the absence of cystine from the diet in amounts adequate to promote normal growth did not affect the composition of the protein.

The analyses of the liver were subject to the difficulties discussed

in connection with the experiments with adult rats. Within these limitations, it appears probable that the constitution of the proteins of the liver was not altered by deficiency of the dietary supply of cystine.

SUMMARY

1. Analyses of fresh muscle, liver, and kidney tissue and of the proteins prepared from these tissues have been made in a series of experiments with young and adult white rats under varying conditions of nutrition.

2. Neither fasting (48 to 72 hours) nor refeeding subsequent to fasting resulted in any significant changes in the composition of muscle and kidney of growing or adult rats or of the proteins of these tissues (nitrogen, sulfur, labile sulfur (determined in muscle proteins only), tyrosine, cystine, tryptophane). The percentages of total nitrogen and sulfur of the livers increased considerably after fasting, but it was not possible to correlate these changes with any alteration in the composition of the proteins of the liver.

3. The sulfur content of all the tissues analyzed was higher in young rats receiving an adequate supply of dietary cystine than in control animals receiving a diet deficient in cystine. No significant differences in the composition of the proteins prepared from these tissues were observed in the two dietary groups. Evidence is presented that the higher sulfur content of the tissues of animals receiving adequate dietary cystine was due to the presence of a larger amount of non-protein sulfur. Whether this sulfur was in the form of cystine or some other non-protein sulfur compound was not determined.

4. The data presented offer no evidence in support of the theory that the composition of the tissue proteins is influenced by dietary factors as maintained by Schenck and Wollschitt (7).

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